

Comparison of Cotinine Salivary Levels Between Smokers, Smokers and Non-Smokers Passive

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ABSTRACT

Background: Cotinine is the predominant metabolite of nicotine which found in tobacco. At present, smoking cigarettes and hookah are on the rise in the Middle East countries; therefore, the number of people exposed to passive cigarette smoking is increasing too. **Objective:** To compare the salivary cotinine level in saliva in smokers, non-smokers, passive smokers. Also to combat the wrong opinion that hookah is not harmful.

Material and methods: In this cross-sectional study, unstimulated salivary samples were collected from 30 female subjects including students and workers at College of Dentistry, Umm Al-Qura university, Makkah. The study participants were divided into 3 groups (each of 10 subjects) aged 19-50 years;

- **First group:** with history of cigarettes or hookah smoking at least one session/day for 20-30 minutes for at least 2 years.
- **Second group:** exposed to cigarette smoke or hookah.
- **Third group:** non-smoker.

TheNicAlert™ (NYMOX pharmaceutical corporation, Canada) nicotine saliva test was used to determine salivary levels of cotinine.

Results: Salivary cotinine level was detected in all study groups with the different levels. The highest level was in a group of smokers while 50% of non-smokers showed level one. There were no significant differences in salivary cotinine levels between hookah, cigarette and both smokers and a significant relation between duration and salivary cotinine level. Also a non-significant relation was found between age and salivary cotinine level.

Conclusion: Salivary cotinine levels were higher in smokers compared to passive smokers and non-smokers, in descending order.

Keywords: cotinine-smokers-smoking-passive smokers-hookah.

INTRODUCTION

Smoking is a world health problem. More than one billion people (1 billion men, 250 million women) smoke in the world resulting in 4.2 million annual deaths. It causes many systemic problem including cancer of lung, oral cavity, pharynx, larynx, oesophagus, pancreas, urinary bladder, and renal pelvis, nasal cavities and nasal sinuses, oesophagus (adenocarcinoma), stomach, liver, kidney (renal cell carcinoma), uterine cervix and myeloid leukaemia, in addition to premature aging^[1]. Tobacco smoke contains over 4800 different chemicals out of which 69 are carcinogens, and several are tumors promoter or cocarcinogens. It has been reported that the exposure duration to tobacco smoke is much more important than the daily number of cigarettes. Quitting as early as possible remains the most powerful factor in reducing the cancer risk^[1]. The presence of carcinoembryonic antigen (CEA) in greater than normal value may indicate the presence of cancer and chronic inflammation, Heavy smoking might also raise blood CEA levels^[1]. In 2008, CDC reported that cigarette

smoking and exposure to second-hand smoke resulted in an estimated 443,000 deaths and 5.1 million years of potential

life lost (YPLL) annually in the United States during 2000-2004^[2].

The main alkaloid of tobacco is Nicotine, which is responsible for its addictive effect. It is easily absorbed from tobacco smoke; in regular smokers, its concentration rises over 6-8 hours during the day. About 70 to 80% of nicotine is metabolized to cotinine which is the main metabolite of nicotine. It's half-life in the body (12-20 hours) is longer than that of nicotine (3-4 hours). Nicotine has been widely used as a specific biomarker of tobacco exposure. Cotinine is suitable for assessment of doses over long periods of time (weeks or months, in hair or nails) or short periods of time (from 1 to 10 days, in urine, plasma, or saliva).

Thus cotinine concentration is feasibly used in epidemiological studies as a biomarker. Cotinine concentration has been used to describe and compare patterns of tobacco consumption in

smokers in different countries to determine if addiction and smoking patterns vary across the population^[3].

Marcela^[4] and Francois^[5] showed that the number of cigarettes smoked is the main determinant of salivary cotinine concentrations.

Youth tobacco use patterns are becoming more complicated in the current marketplace where tobacco products are various. Although youth use of conventional cigarettes is on the descend, use of nonconventional products is rising, and there may be a rising trend for using cigarettes with another product. Between 2000 and 2012, there was an overall decline in tobacco use for high school students among those who reported using only conventional cigarettes. Over the same period, use of electronic cigarettes and water pipe (or hookah) has grown. Youth prevalence of electronic cigarette ever, current, and use with traditional cigarettes doubled between 2011 and 2012, with almost 10% of students who have used electronic cigarettes having never used conventional cigarettes. Current use of hookah among high school students also increased during this time, whereas use of other products, including bidis (small brown cigarettes wrapped in a leaf), kreteks (clove cigarettes), and dissolvable tobacco products, significantly decreased^[6].

Differences in health risks and the level of nicotine associated with these tobacco products make it important for policymakers and regulatory agencies interested in protecting the public health to understand how these products are used, especially among youth because most individuals first experiment with tobacco use during this period^[6]. Unfortunately, the marked decline in cigarette use has been accompanied by a relatively recent increase in adolescent (i.e., 11–18-years-old) and young adult (i.e., 18–25-years-of-age) use of new and emerging alternative tobacco products (ATPs).

Whereas the total consumption of cigarettes in the U.S. has decreased 33% between 2000 and 2011, the consumption of non-cigarette combustible tobacco products overall has increased an alarming 100% over the same time period. Use of many ATP types among adolescents is increasing due to a variety of influences, including availability; widespread unsubstantiated perception of safety; lack of or non-implemented regulations surrounding their use; reduced cost and easy accessibility; and, attractive smell and taste^[7].

The term "smoking" refers to the intentional inhalation of tobacco smoke by a smoker. The term "passive smoking" refers to, the

unintentional inhalation of tobacco smoke by non-smokers introduced into the atmosphere by smokers. The term "passive smoking" introduced to the world in the 1970s; till then, researcher continues to find the evidence linking environmental tobacco smoke with illness, and great work has been undertaken to find ways of measuring the extent of tobacco smoke exposure in non-smokers. Of these, measurement of nicotine and cotinine levels in the body has received the most attention^[8].

It has been estimated that deaths due to tobacco smoke will increase to 10 million a year by 2020, with more deaths in developing countries compared to developed countries^[9]. Recent research has shown that smoking cigarette and hookah, is the most important etiologic factors for oral squamous cell carcinomas^[10].

Hookah (or water pipe) shisha (tobacco or non-tobacco herbal, combustible materials used to smoke hookahs) use in the U.S. has increased rapidly over the past two decades, due to the increased immigration of Middle Eastern and South Asian populations, where hookah smoking originated and considered as part of their cultural identity. Although little is known about the diffusion of hookah use to other racial/ethnic groups in the U.S., its use nationwide is rapidly increasing (particularly, in urban areas), while regulations for use are limited^{[11][12]}.

Smoking hookah does not reduce the risk of tobacco exposure and it's potentially harmful metabolites on health^[13]. Following a single run of hookah smoking, plasma, saliva and urinary nicotine and cotinine concentration increased to high values. thus hookah may not be an innocent habit, as people believe^[14].

As children showed a higher exposure compared with adults the health hazards association with passive smoking might have a more serious effect in children. Children are unable to grumble and unable to remove themselves from tobacco smoke exposure. Thus, parents should make sure that their children live in a smoke-free environment. Each country should take all necessary statutory and regulatory measures which prevent smoking in public places and the home environment even if it requires sacrifices by the parents^[15]. The cotinine concentrations are significantly greater in unstimulated than in stimulated saliva, because of alteration in flow rate will change the PH^[16]. African American smokers generally have higher plasma cotinine levels than Caucasian smokers^[17]. And Males have higher plasma

cotinine levels than females^[18]. These systematic differences in cotinine levels refer to variation in CYP2A6 activity^[19].

MATERIALS AND METHODS

The present cross-sectional study carried out in Umm Al-Qura university students (Faculty of Dentistry) and workers, 30 subjects were included in the study in three group: smokers, passive smokers, nonsmokers.

A standard questionnaire used to collect demographic and smoking behavioral data at the same appointment of saliva collection.

Information on the following items obtained by the questionnaire: demographic data (age, gender), the average number of cigarettes smoked daily or the average daily consumption of hookah, the smoking or exposure in the last 24 hours, the use of other forms of tobacco (cigars, pipe, chewing tobacco, electronic cigarettes, nicotine replacement therapy).

The inclusion criteria content of female students and workers in Umm Al-Qura University (faculty of dentistry) in Mecca, submitting an informed consent form, age range of 19-50 years, a history of smoking cigarettes or hookah at least one session a day for 20-30 minutes for at least 2 years (10 subjects), individuals exposed to cigarette smoke or hookah at home or workplace (10 subjects), and a non-smoker who did not smoke cigarettes or hookah (10 subjects). Exclusion criteria content of subjects with any systemic diseases, oral lesion, use of medications and smoking any other type of tobacco.

A collection of the unstimulated saliva samples performed using standard method (funnel, cap, and tube container) provided by NicAlert™ Saliva strip tests (NYMOX Pharmaceutical Corporation, QC Canada).

The tube containers with the saliva samples stored at -20C before the analysis of the cotinine level (at this temperature saliva is stable for biomarkers evaluation for minimum 3 months)^[20].

Semi-quantitative immunoassay technology, in the form of rapid test strips, offers a less cost and less time-consuming alternative to other methods of confirming self-reported smoking status, such as gas chromatography-nitrogen phosphorus detection (GC).

Regrettably, information on the validity and reliability of some test strips in urine and saliva samples is not always available. The use of NCTS is a valid and reliable method, compared with GC, to test saliva samples for verification of smoking status^[21]. NicAlert Saliva test strip zones range

from zone 0 (0-10 ng/mL) to zone 6 (>1000 ng/mL).

The cutoff concentration for the NicAlert™ test (an immunochromatographic assay using a monoclonal antibody), indicating a positive result, was 10 ng/mL (zones 1-6). The salivary cotinine concentration and its interpretation for each level of the NicAlert™ are shown in the table 1.

Table1: cotinine equivalent for each level

Level on strips	Cotinine equivalent
0	1-10 ng/ml
1	10-30 ng/ml
2	30-100 ng/ml
3	100-200 ng/ml
4	200-500 ng/ml
5	500-2000 ng/ml
6	2000+ ng/ml

The study was done after approval of ethical board of Umm Alqura university.

RESULTS

The results were processed by using SPSS program by using:

- 1- Descriptive statistics (mean, SD, minimum and maximum value, frequencies and percentage). To describe the variables of the study.
- 2- ANOVA test followed by Scheffe test was used to compare between the three groups in salivary cotinine levels.
- 3- Pearson correlation coefficient was used to study the relation between cotinine levels and age and duration.

Table (2) descriptive statistics of the smoker group attributed to age- duration- level

Sample		Age	Duration	Cotinine Level
Smoker	mean	22.9	4.8	5.2
	SD	1.4	2.09	1.3
	Minimum	20	2	30-100 level
	Maximum	25	8	500- 100 level 6
	p- value	0.001	0.001	0.001

With respect to table 2 for smoker group, the results showed that:

- 1- The mean of ages in this group was 22.9, with standard deviation mean of 1.4 and minimum age was 20 and maximum age was 25.
- 2- The mean of the duration of smoking in this group was 4.8 year, with standard deviation of

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2.09 and minimum duration was 2 and maximum duration was 8.

3- The mean of the cotinine level in this group was 5.2, with standard deviation of 1.3 and the minimum level was 30-100 which indicates to level 1 and maximum level was 500-1000 which indicates to level 6.

Table (3) descriptive statistics of the passive group attributed to age- duration- level.

Sample		age	Duration	Cotinine Level
Passive	mean	25.6	16.5	2.1
	SD	4.57	7.13	0.8
	Minimum	22	3	0-10 level 0
	Maximum	32	25	(100-200) level3
	p- value	0.001	0.001	0.001

With respect to table 3 for a passive group, the results showed that:

1- The mean of ages in this group was 25.6, with standard deviation mean 4.57 and the minimum age was 22 and the maximum age was 32.

2- The mean of the duration of smoking in this group was 16.5 year, with standard deviation 7.13 and minimum duration was 3 and maximum duration was 25.

3- The mean of the cotinine level in this group was 2.1, with standard deviation 0.8 and the minimum level was 0-10 which indicates to level 0 and the maximum level was 100-200 which indicates to level 3.

Table (4) descriptive statistics of the non-smoker group attributed to age- duration- level

Sample	age	Cotinine Level
Non smoker	mean	24.8
	SD	6.1
	Minimum	20
	Maximum	37
	p- value	0

With respect to table 4 for Nonsmoker group, the results showed that:

1- The mean of ages in this group was 24.8, with standard deviation mean 6.1 and minimum age was 20 and maximum age was 37.

3- The mean of the cotinine level in this group was 1.5, with standard deviation 0.5 and the minimum level was 0-10 which indicates to level 0 and the maximum level was 10-30 which indicates to level 1.

Table (5) distribution of salivary cotinine levels in the three groups

Level cotinine	Smoker	Passive	Non smoker	Total	Percentage
Level 0 (0-10)	0	2	5	7	23.3%
Level 1 (10-30)	0	6	5	11	36.7%
Level 2 (30-100)	1	1	0	2	6.7%
Level 3 (100-200)	3	1	0	4	13.3%
Level 4 (200-500)	0	0	0	0	0%
Level 5 (500-1000)	5	0	0	5	16.7%
Level 6 (>1000)	1	0	0	1	3.3%

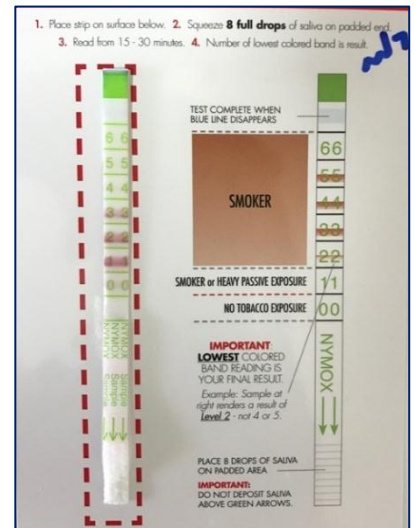
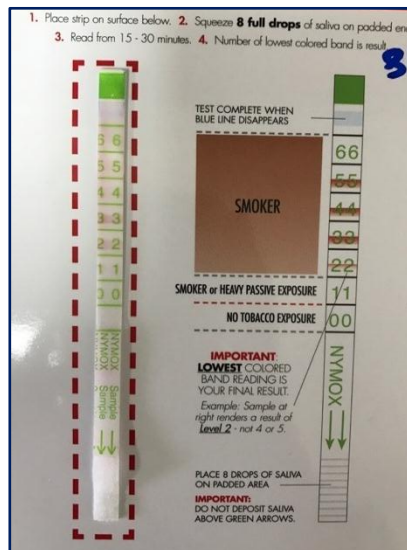
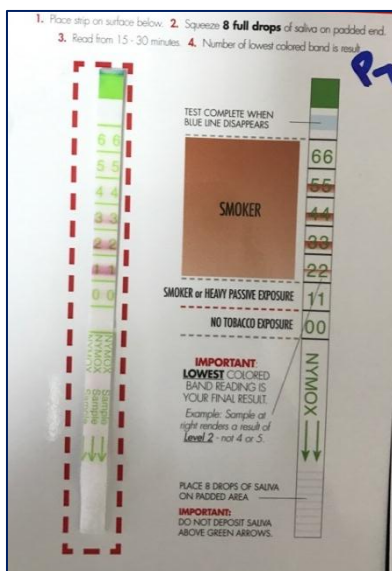
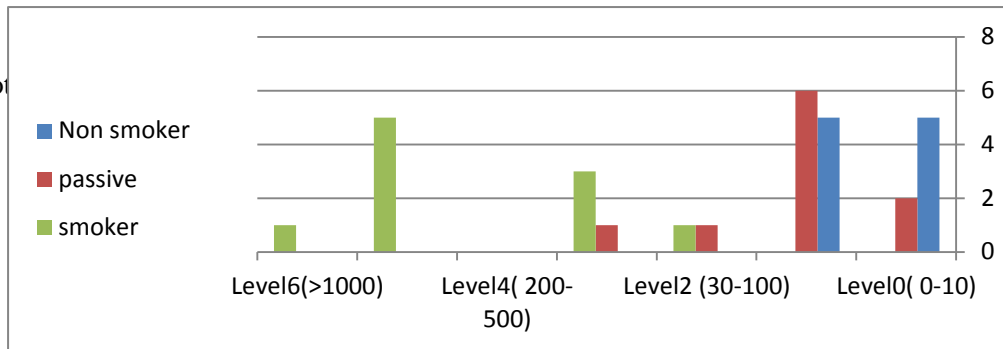


Fig. 1: Cot**Fig 2: distribution of salivary cotinine levels in the three groups**

With respect to a table 5, fig1 and fig2 for cotinine levels in the three groups the results showed that:

- 1-The most frequently salivary cotinine level was level 1 by 11 members with a percentage 36.7%, the passive group includes 6 members, while non-smoker group involved 5 members.
- 2- The second salivary cotinine level was level 0 by 7 members with a percentage (23.3%), the passive group includes 2 members, while non-smoker group involved 5 members.
- 3- The third salivary cotinine level was level5 by 5 members with a percentage 16.7% in the smoker group.
- 4- The fourth salivary cotinine level was level3 by 4 members with a percentage 13.3%, the passive group include 1 member, while smoker group involved 3 members.
- 5- The fifth salivary cotinine level was level2 by 2 members with a percentage 6.7%, the passive group include 1 member, while smoker group involved 1 member.
- 6- The sixth salivary cotinine level was level6 by 1 member with a percentage 3.3 % all of them in the smoker group. From the previous results, we can say that the highest salivary level was recorded in smokers group followed by passive smoker's group.

Table (6) ANOVA test to compare between the three groups in salivary cotinine levels

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	78.867	2	39.433	2.588	.001
Within Groups	25.000	27	.926		
Total	103.867	29			

From the previous table 6 the value of F was 42.588 and p-value 0.000 , it is obvious that there is a statistically significant differences between the three groups in the salivary cotinine levels where the(p-value < 0.05) To determine the direction of the differences, Scheffe test was used for post-comparisons, and the results indicated that the differences between smoker groups and passive groups were in favor of smoker groups, and between smoker group and non-smoker group in favor of smoker group too:

Table (7) distribution of smoking type in the smokers group

Smoking type	N	Percent
Hookah	5	50%
Cigarette	1	10%
Both	4	40%

With respect to table 7 for cotinine concentration in the smoker group the results showed that: there was 5 persons with percentage 50% used hookah, while 1 person with percentage 10% used cigarette, and 4 persons with percentage 40% used both of them.

Table (8) the correlation between age and salivary cotinine level

	value	df	p-value
Pearson correlation coefficient	-0.226	30	0.229

With respect to a table 8 the value of Pearson correlation coefficient was (-.226) a so there is a non-significant relation between age and salivary cotinine level, as (p-value > 0.05).

Table (9): the correlation between duration and salivary cotinine level

	value	df	p-value
Pearson correlation coefficient	-.646*	20	0.002

With respect to a table 9 the value of Pearson correlation coefficient was (-0.646) at p-value (0.002), as the p-value (<0.05) so we can say there is a significant relationship between duration and salivary cotinine level.

DISCUSSION

Cotinine is an alkaloid and is an anagram of the term nicotine and because the durability of it is 20 hours compared with the durability of nicotine which is 2 hours. So, salivary cotinine level is used as a biomarker to identify cigarette smokers, hookah smokers, and passive smokers^[22]. From our results, the mean value of ages of smokers was 22.9, passive smokers were 25.6 and non-smokers was 24.4. The cotinine level was 5.2 in the smokers group, 2.1 in the passive group and 1.5 in the non-smokers group. In the present study, no significant relationship was found between the salivary cotinine level and exposure to tobacco and age. That was in consistency with **Asha and Dhanya**^[23], they worked on 75 tobacco chewers aged between 20 to 50 years and found no significant relationship between the level of cotinine and age of patients

Seifi et al. did not show a relationship between salivary cotinine levels in smokers and exposure to tobacco smoke and age^[24]. **Figueiredo et al.**^[25] have shown that cotinine metabolism is not affected by age, while others, **Campuzano et al.**^[26], and **Lubin et al.**^[27], have found a significant association.

In this study, there was a non-significant relation between cotinine concentration and duration. That was inconsistent with that of **Figueiredo et al.**^[25], who had found no significant association between cotinine concentration and duration, although among those reporting 1–20 cigarettes in the past 24 hours, a pattern of higher mean levels of cotinine with longer duration of smoking (>25 years) was seen. Although in a study was done by **Etter et al.**^[28], they reported that a positive association between cotinine concentration with duration, which, however, disappeared after multiple adjustments. In our study the percent of hookah smokers was 50%, in cigarette smokers was 10% and both hookah and cigarette smokers were 40%. In the smokers group, the mean of the cotinine level was 5.2 with standard deviation 1.3 and minimum level was 30-

100 and the maximum level indicated to level 6 (>1000).

While in the passive smokers group the mean of the cotinine level was 2.1 with standard deviation 0.8 and the minimum level was 0-10 and maximum level indicated to level 3 (100-200). Our results were supported by a study by **Jaakkola et al.**^[29], they noticed increased in salivary cotinine level almost 5.5 ng/ml for every extra cigarette smoked. In the non-smokers group, the mean of the cotinine level was 1.5 with standard deviation 0.5 and minimum level was 0-10 and the maximum level indicated to level 0 (0-10).

The results of the present study showed the highest salivary cotinine levels in smokers, passive smokers followed non-smokers in descending order. In the present study, no reported results were found to compare the salivary cotinine level in hookah and cigarette smokers. Though a higher level of salivary cotinine levels in hookah smokers in comparison to cigarette smokers has been confirmed in other studies.

The results of the present study were in agreement with those of **Rabiei et al.**^[30] and **Primack et al.**^[31].

Both studies showed an increase in salivary cotinine level between non-smokers and passive smokers indicating the importance of this problem and avoiding environments that might expose individuals to tobacco smoke, which might increase cotinine and nicotine levels in the body, resulting in dangerous complications.

NicAlert strips appear to be a valid measure of determining smoking status smokers and passive smokers although not of absolute cotinine concentration. With minimal training and equipment required, and easy interpretation, the results are available in 20-30 minutes. A potential limitation for NicAlert strips is that their cost can appear relatively high^[32].

The present study's limitations included its reliance on information provided by subjects with regard to the independent variables. More studies are needed to link other variables with salivary cotinine level such as the sex of subjects or/and their body weights.

CONCLUSION

- Salivary cotinine levels were higher in smokers compared to passive smokers and non-smokers, in descending order.
- The salivary concentration of cotinine can be used to estimate nicotine intake.

- Public enforcement and awareness are urgently needed to convey information regarding risks associated with hookah use to adolescents and teenagers and combat the wrong opinion that hookah smoking is not harmful.
- This study strengthens the evidence for the benefits of a smoking ban in public places.
- However, studies on larger scale population and with different duration of smoking are recommended to validate the results.

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